Effects of High Glucose Concentrations and Epalrestat on Sorbitol and *myo*-Inositol Metabolism in Cultured Rabbit Aortic Smooth Muscle Cells

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To clarify the relationship between abnormality of sorbitol and/or *myo*-inositol metabolism caused by hyperglycemia and diabetic macroangiopathy, we investigated the effects of high glucose concentrations and epalrestat, an aldose reductase inhibitor, on the metabolism of sorbitol and *myo*-inositol in cultured rabbit aortic smooth muscle cells. In cells incubated in the presence of 30 mM glucose for 72 h, the sorbitol content increased ~4.5-fold, and the *myo*-inositol level decreased by 55% compared with control values. Kinetic analysis of high-affinity *myo*-inositol uptake suggested that smooth muscle cells exposed to high glucose concentrations exhibited a noncompetitive type of inhibition characterized by ouabain-sensitive, energy-dependent active transport. Epalrestat blocked glucose-induced changes in sorbitol and *myo*-inositol metabolism, suggesting that these changes were caused by the accumulation of sorbitol in the cells. These metabolic changes may impair function of smooth muscle cells, contributing to the pathology of diabetic atherosclerosis, especially Mönckeberg's calcific medial sclerosis. The use of an aldose reductase inhibitor may prevent these glucose-induced changes. *Diabetes* 42:1594–1600, 1993

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Received for publication 29 December 1992 and accepted in revised form 17 June 1993.

ARI, aldose reductase inhibitor; DMEM, Dulbecco's modified Eagle's medium; FCS, fetal calf serum; PBS, phosphate-buffered saline; IgG, immunoglobulin G; FITC, fluorescein isothiocyanate; HPLC, high-performance liquid chromatography; BSA, bovine serum albumin.

Macrovascular complications related to atherosclerosis are the principal causes of mortality in a diabetic population (1–3). Atherosclerosis tends to occur at an earlier age and with greater severity in individuals with diabetes than in the general population (4).

Late complications of diabetes mellitus are mainly related to the involvement of arterial walls of both small and large vessels and of the peripheral nervous system. The presence of such complications in almost all types of diabetes mellitus suggests that they have a common pathogenetic mechanism, which is manifested by high blood glucose levels and related alterations (5,6). Hyperglycemia itself leads to some metabolic abnormalities, such as increased polyol pathway activity (7). Previous studies have suggested that abnormal glucose metabolism, the accumulation of intracellular sorbitol, and a decrease in *myo*-inositol content may also contribute to late complications of diabetes (8,9). This abnormal metabolism, which has been confirmed in experimental models (8,9), plays a key role in the development of diabetic complications.

Smooth muscle cells constitute a major element of the arterial wall and play an important role in the development of atherosclerosis. The proliferation and migration of smooth muscle cells are key events in the initiation and perpetuation of fibrous plaque, the pathognomonic lesion of atherosclerosis (10,11). The existence of a polyol pathway in the arterial wall (12–14) suggests that hyperglycemia may lead to the accumulation of sorbitol within smooth muscle cells, contributing to their dysfunction. Although sorbitol may impair the function of various cells through its osmolar effect, the depletion of NADPH, or other effects, caused by the accumulation of sorbitol in tissues may be associated with depletion of *myo*-inositol, resulting in suppression of phosphoinositide turnover and subsequent metabolic abnormalities (15,16). We

investigated this hypothesis by measuring sorbitol and *myo*-inositol levels in rabbit aortic smooth muscle cells cultured in the presence of various glucose concentrations. We also investigated the effects of glucose on *myo*-inositol uptake by smooth muscle cells to determine the mechanisms of changes in *myo*-inositol uptake in smooth muscle cells. We also examined the effects of epalrestat, an ARI (9), on glucose-induced changes.

RESEARCH DESIGN AND METHODS

Culture of smooth muscle cells. Rabbit arterial smooth muscle cells were cultured as described previously by Ross (17). Explants were obtained from the intima-media segments of the thoracic aorta of male New Zealand rabbits, weighing 1500–2000 g, that were maintained on normal laboratory chow. They were anesthetized by the intravenous injection of sodium pentobarbital (50 mg/kg body wt) and killed by blood loss. The intima-media segments were prepared under sterile conditions after adherent tissue and adventitia were removed. Approximately 15 explants (1 mm^2) were placed in 25-cm^2 polystyrene tissue culture flasks (Falcon 3013, Becton Dickinson, Lincoln Park, NJ). After the explants had attached to the bottom wall of the flasks, 1 ml of growth medium consisting of DMEM, supplemented with 100,000 U/L of penicillin G, 50 mg/L of streptomycin, 14.9 mM of sodium bicarbonate, and 10% FCS was added to each flask. The culture flasks were incubated in a humidified incubator at 37°C in 5% CO_2 and 95% room air. The initial outgrowth of the smooth muscle cells was observed by phase-contrast microscopy in 1 wk. Half of the medium was exchanged for the fresh medium 3 times/wk.

When the cells became confluent, the explant segments were removed from the flasks. The cells in the flasks were dissociated by the addition of PBS containing 500 mg/L of trypsin and 0.5 mM of disodium ethylenediaminetetraacetate. Cells were then washed with growth medium, centrifuged (40 *g*), and diluted with the growth medium; ~5 × 10^4 cells in 1 ml of medium were seeded in culture dishes 60 mm in diameter (Falcon 3002, Becton Dickinson).

Phase-contrast microscopy showed that cultured rabbit aortic smooth muscle cells grew predominantly in multiple, overlapping layers with hills and valleys. Electron microscopy showed that the confluent smooth muscle cells contained rich, thin filaments, several thick filaments, dense body, rough endoplasmic reticulum, free ribosomes, mitchondrias, and many vesicles. Smooth muscle cells produced an extracellular matrix. Both phase-contrast and electron microscopy findings were consistent with the morphological characteristics of smooth muscle cells described by Ross (17). Subcultured smooth muscle cells from the second passage were stained immunofluorescently with rabbit anti-muscle actin (purified from chicken gizzards) IgG antigen (Biomedical Technologies, Stoughton, MA) combined with FITC. Smooth muscle cells exhibited numerous fluorescent cables. A fantastic assortment of fibers and diffuse cytoplasmic fluorescence were observed. Broad and fine fibers were extended along the cell axis. These findings were consistent with the characteristics of smooth muscle cells described by Chamley et al. (18).

Cells were repeatedly subcultured until a sufficient volume was produced. The third passage was used for all experiments. Despite several subcultures, homogeneity of the cells in each cell series was not established. The culture dishes that appeared to be contaminated by fibroblasts were excluded from the study.

Measurement of intracellular sorbitol and *myo*-inositol content. Arterial smooth muscle cells present in culture dishes after cells reached confluence were washed and incubated in a humidified incubator at 37°C with DMEM containing 10% FCS, pH 7.4, with 5.5–30 mM glucose and with or without epalrestat for 1–72 h. At the end of the incubation period, cells were immediately washed 3 times with cold saline and homogenized in 2 ml of cold water. Galactitol used as an internal standard was added to each sample, and proteins were precipitated with ethanol (70% of final volume) and eliminated by centrifugation at 12,000 *g* for 15 min. After the supernatants were collected, the sample was lyophilized. To obtain derivatives of sugars and polyols, 250 μl of pyridine and 500 μl of phenylisocyanate were added to each lyophilized sample according to the method described by Dethy et al. (19). The sample reaction was stopped, and the derivatives were formed by incubating the solution at 55°C for 1 h in a water bath with mechanical shaking. After incubation, the sample reactants were cooled, and 250 μl of methanol was added to eliminate excess phenylisocyanate. The solutions were then diluted twice with pyridine to reduce interference caused by the absorption of the ligands.

Polyol derivatives were then applied to a column (Nucleosil 5C18MN, Macherey-Nagel, Düren, Germany), eluted with a mixture of 60% acetonitrile and 40% 10 mM K_2HPO_4 in water at a flow rate of 1.2 ml/min using HPLC (SPD-6A, Shimadzu, Kyoto, Japan), and measured at 240 nm.

Measurement of *myo*-inositol uptake. *myo*-Inositol uptake experiments were performed in 24-well plates (Falcon 3047, Becton Dickinson) on a waterbath shaker after cells were incubated with *myo*-[2-^{3}H]inositol (92.5 kBq/ml) in a buffer containing 150 mM NaCl, 2 mM $CaCl_2$, 20 mM HEPES (buffer 1), pH 7.4, with 1% BSA, the required concentrations of unlabeled *myo*-inositol (final concentration: 1–1000 μM), glucose (0–40 mM), ouabain, and various drugs including ARIs (epalrestat and statil), hormones, and substrates. Uptake was terminated by removing the incubation medium, cooling the culture plates to 0°C immediately, and rapidly washing the cells 3 times with cold buffer 1. The cells were harvested, and the radioactivity was assayed in a liquid scintillation counter.

To examine the effect of sodium ion on *myo*-inositol uptake, we substituted LiCl (buffer 2) for the NaCl in buffer 1. The sodium-dependent *myo*-inositol uptake was calculated by subtracting uptake in the absence of sodium concentration from uptake in the presence of high sodium concentrations.

Materials. Glucose and the other hexoses were obtained from Katayama Chemical (Osaka, Japan). DMEM and FCS were obtained from Gibco (Life Technologies,

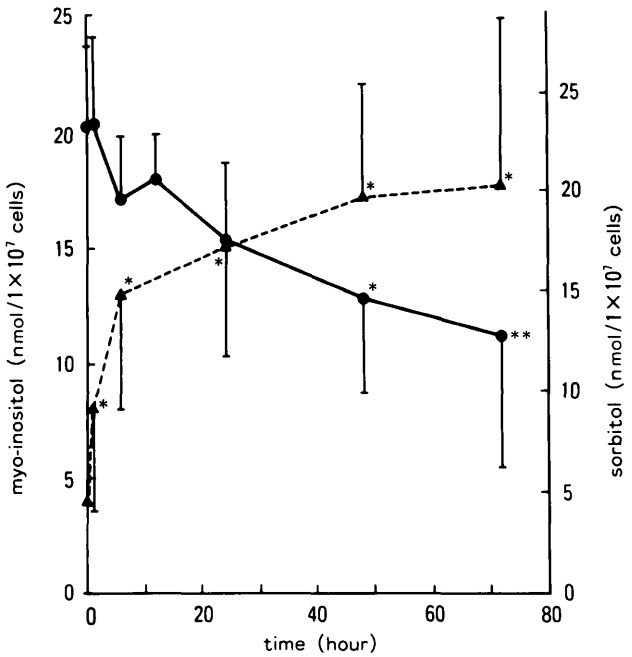

FIG. 1. Effects of glucose on intracellular sorbitol and *myo*-inositol content. Smooth muscle cells were incubated for 0–72 h in 30 mM glucose. Cells were periodically analyzed for sorbitol (▲---▲) and *myo*-inositol (●——●) content. Data are means ± SD of 6–9 experiments. *$P < 0.05$; **$P < 0.01$ vs. control (0 h).

Grand Island, NY). Trypsin was purchased from Difco (Detroit, MI). *myo*-[2-^{3}H]Inositol was purchased from NEN-Du Pont (Boston, MA). Epalrestat and statil were gifts from Ono Pharmaceutical (Osaka, Japan) and ICI (Osaka, Japan), respectively. The remaining chemicals and reagents were purchased from Sigma (St. Louis, MO) and were of the highest grade available.

Statistical analysis. Data are expressed as means ± SD. The difference between paired samples in each group of paired experiments was calculated using the paired Student's *t* test. Between group differences were analyzed using the unpaired Student's *t* test. A level of $P < 0.05$ was accepted as statistically significant.

RESULTS

Intracellular sorbitol and *myo*-inositol content in smooth muscle cells. Smooth muscle cells were incubated in media containing 5.5–30 mM glucose. The supplemental growth conditions had no effect on cell growth, as determined by final cell number after 72 h. In addition, no changes in the gross morphological appearance of the cells cultured in the supplemental conditions were observed with light microscopy.

Smooth muscle cells incubated in the presence of 30 mM glucose for 72 h contained significantly more sorbitol than control cells (Fig. 1). Because the increase in sorbitol occurred rapidly, the difference was significant after 1 h of incubation with 30 mM glucose. In contrast, intracellular *myo*-inositol levels decreased significantly after cells were cultured in 30 mM glucose. Because the decrease in *myo*-inositol occurred relatively slowly, the difference became significant after incubation for 48 h. The magnitudes of the increase in intracellular sorbitol

TABLE 1
Effects of epalrestat on sorbitol and *myo*-inositol content

Glucose (mM)	Epalrestat (mM)	Sorbitol content (nmol/1 × 10^7 cells)	*myo*-Inositol content (nmol/1 × 10^7 cells)
5.5	0	4.6 ± 4.5	20.4 ± 4.2
5.5	0.1	4.7 ± 4.7	21.3 ± 4.7
30	0	20.3 ± 7.2*	11.2 ± 6.6†
30	0.1	6.2 ± 5.8	19.1 ± 6.6

Data are means ± SD of 6–9 experiments.
*$P < 0.05$ vs. 5.5 mM glucose, 5.5 mM glucose plus 0.1 mM epalrestat, and 30 mM glucose plus 0.1 mM epalrestat.
†$P < 0.01$ vs. 5.5 mM glucose, 5.5 mM glucose plus 0.1 mM epalrestat, and 30 mM glucose plus 0.1 mM epalrestat.

levels and decrease in *myo*-inositol contents depended on the extracellular concentration of glucose. After cells were exposed to 30 mM glucose for 72 h, the intracellular sorbitol content increased ~4.5-fold compared with the control level, and the *myo*-inositol content decreased by ~55%. The addition of 0.1 mM epalrestat, an ARI, almost completely blocked the effect of glucose on both intracellular sorbitol and *myo*-inositol (Table 1).

Kinetic analysis of *myo*-inositol uptake and effect of sodium ion. *myo*-Inositol uptake occurred in a nearly linear fashion for at least 90 min (Fig. 2). To further characterize the relationship between varying *myo*-ino-

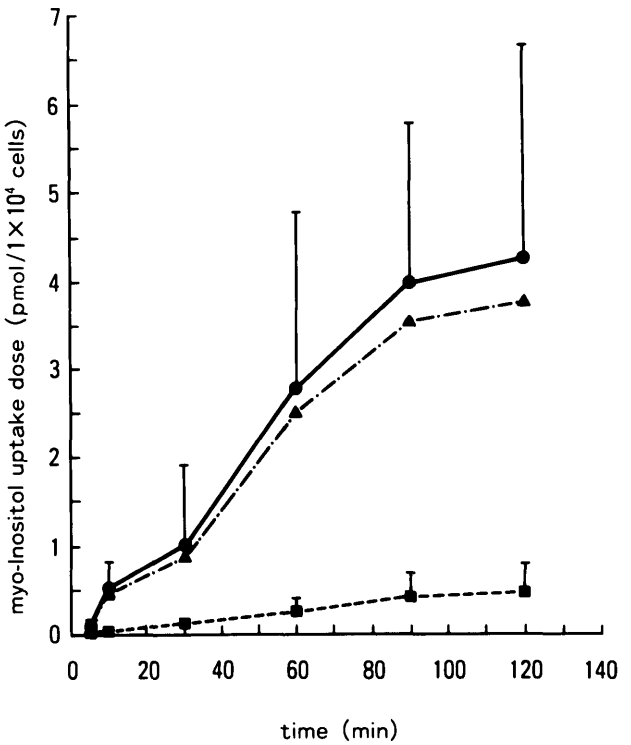

FIG. 2. Time course of *myo*-inositol uptake by smooth muscle cells cultured with 150 mEq/L sodium (buffer 1) (●——●) or without sodium (buffer 2) (■---■). Cells were sequentially preincubated with either buffer 1 or buffer 2 (15 min), the same buffer containing 50 μM *myo*-inositol, and then buffer containing *myo*-[2-^{3}H]inositol for the time indicated. Sodium-dependent uptake (▲–·–·–▲) was calculated as the difference between uptake in the presence and absence of sodium at each point. Glucose concentration was 0 mM. Data are means ± SD of 6–24 experiments.

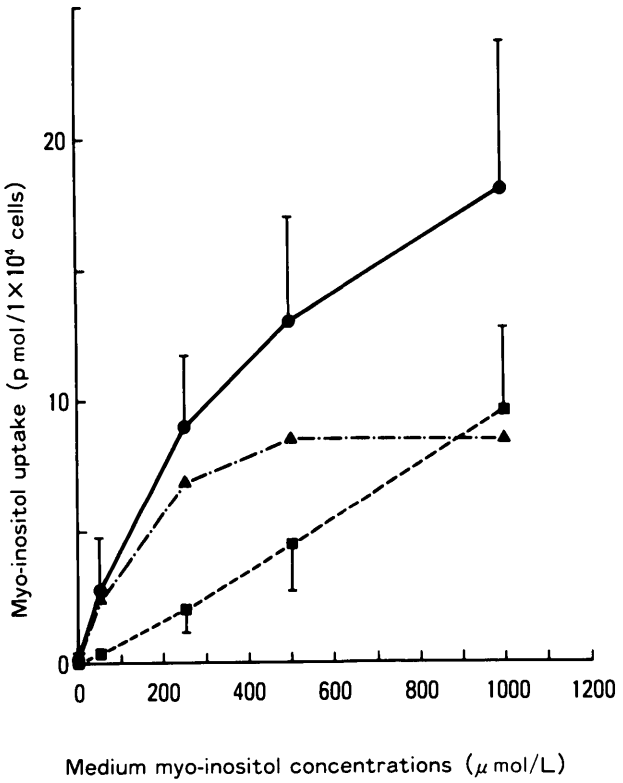

FIG. 3. Intracellular *myo*-inositol uptake as function of *myo*-inositol concentrations in the presence or absence of external sodium ion. Sodium concentrations of media were 150 mEq/L (buffer 1) (●——●) or 0 mEq/L (buffer 2) (■---■). Sodium-dependent uptake (▲-·-·-▲) was calculated as the difference between uptake in the presence and absence of sodium at each point. Glucose concentration was 0 mM. Results are the mean value for 60 min. Data are means ± SD of 6–24 experiments.

sitol concentrations and *myo*-inositol uptake, smooth muscle cells were incubated for 60 min in *myo*-inositol concentrations ranging from 1 to 1000 μM (Fig. 3). The uptake rate increased with increasing concentrations of *myo*-inositol, but the slope of the curve was much lower at high concentrations of *myo*-inositol. In parallel incubations with buffer 2, the sodium ion in the medium was replaced by lithium ion. The uptake rate increased linearly with increasing *myo*-inositol concentrations. These findings suggest that there were at least two distinct uptake mechanisms, a low-affinity uptake, which was unsaturable, and a high-affinity saturable uptake, which was sodium dependent. The sodium-dependent high-affinity uptake was calculated by subtracting uptake in the presence of buffer 2 from uptake in the presence of buffer 1. Kinetic data calculated for the sodium-dependent uptake fitted the Lineweaver-Burk transformation of the Michaelis-Menten equation. The resting curve showed an apparent K_m of 0.17 mM and a V_{max} of 83.3 pmol *myo*-inositol · 1 × 10^4 cells^{-1} · 60 min^{-1}.

Effects of glucose, other hexoses, hormones, and ARIs on *myo*-inositol uptake. Glucose inhibited *myo*-inositol uptake in a dose-dependent manner (Tables 2 and 3). Analysis of this inhibition with the use of Dixon plots suggested that inhibition was noncompetitive, and K_i was determined on 45 mM (Fig. 4*A*). In a Dixon plot

TABLE 2
Effects of glucose and epalrestat on *myo*-inositol uptake

Glucose (mM)	Epalrestat (mM)	*myo*-Inositol uptake (pmol/1 × 10^4 cells)	Change from control (%)
0	0	2.78 ± 2.01	100
	0.1	2.72 ± 1.81	98
5	0	2.49 ± 1.62	89
	0.1	2.57 ± 2.01	93
20	0	1.61 ± 1.13*	58
	0.1	2.83 ± 2.11	102
40	0	1.57 ± 1.21*	56
	0.1	2.65 ± 1.02	95

Data are means ± SD of 12–24 experiments.
*$P < 0.05$ vs. 0 mM glucose, 0 mM glucose plus 0.1 mM epalrestat, 5 mM glucose, 5 mM glucose plus 0.1 mM epalrestat, 20 mM glucose plus 0.1 mM epalrestat, and 40 mM glucose plus 0.1 mM epalrestat.

analysis using the combination with high glucose and epalrestat, we failed to observe results similar to those seen in Fig. 4*A* (Fig. 4*B*). Epalrestat almost completely reversed the inhibitory effect of glucose on *myo*-inositol uptake (Table 2). A similar effect was also observed by statil, another ARI (Table 3).

As shown in Tables 4, 5, and 6, the addition of glucose analogs L-glucose, 3-*O*-methyl-D-glucose, or 2-deoxy-D-glucose did not reduce *myo*-inositol uptake. Epalrestat and the combination of epalrestat and the hexoses did not influence *myo*-inositol uptake. Fructose, a member of the aldohexose family that occupies one corner of the sorbitol pathway, reduced *myo*-inositol uptake, but not significantly (Table 7). Insulin, glucagon, and epinephrine had no inhibitory effect on *myo*-inositol uptake. However, ouabain, a selective Na^+/K^+-ATPase inhibitor, markedly reduced *myo*-inositol uptake at concentrations ranging from 0.1 to 1 mM. A 1-mM concentration of ouabain inhibited *myo*-inositol uptake by 52% compared with control. *myo*-Inositol uptake was reduced 27% by 10 μM cytochalasin B, a potent blocker of glucose transport, which was not statistically significant.

DISCUSSION

Our results showed that glucose induced sorbitol accumulation and decreased *myo*-inositol content in cultured

TABLE 3
Effect of glucose and statil on *myo*-inositol uptake

Glucose (mM)	Statil (mM)	*myo*-Inositol uptake (pmol/1 × 10^4 cells)	Change from control (%)
0	0	3.29 ± 0.62	100
	0.1	3.51 ± 0.40	107
5	0	3.82 ± 0.86	116
	0.1	2.99 ± 0.57	91
20	0	1.88 ± 0.48*	57
	0.1	2.86 ± 0.31	87
40	0	1.54 ± 0.49*	47
	0.1	2.64 ± 0.51	80

Data are means ± SD of 3–6 experiments.
*$P < 0.05$ vs. 0 mM glucose, 0 mM glucose plus 0.1 mM statil, 5 mM glucose, 5 mM glucose plus 0.1 mM statil, 20 mM glucose plus 0.1 mM statil, and 40 mM glucose plus 0.1 mM statil.

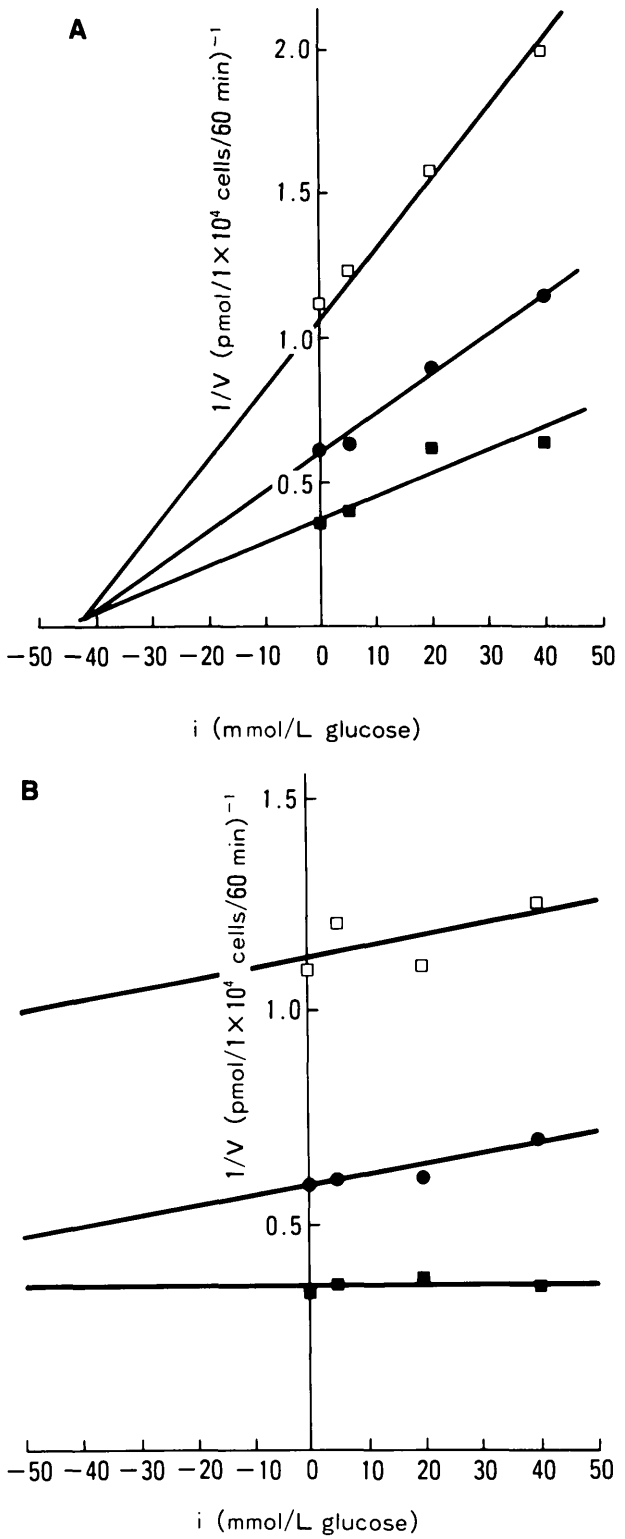

FIG. 4. Dixon plot analysis for glucose inhibition of *myo*-inositol uptake in the absence (*A*) or presence (*B*) of epalrestat by smooth muscle cells. Cells were incubated for 60 min at 5 (□—□), 10 (●—●), and 50 (■—■) μM *myo*-inositol concentrations. Each point is the mean of 6–24 experiments.

rabbit aortic smooth muscle cells. These findings reflect in vivo findings in the diabetic rat peripheral nerve (7,20–23) and glomeruli of kidney (24,25) and in vitro findings in cultured bovine aortic endothelial cells (26)

TABLE 4
Effects of L-glucose and epalrestat on *myo*-inositol uptake

L-glucose (mM)	Epalrestat (mM)	*myo*-Inositol uptake (pmol/1 × 10^4 cells)	Change from control (%)
0	0	2.78 ± 2.01	100
	0.1	2.72 ± 1.81	98
5	0	2.70 ± 2.15	97
	0.1	2.80 ± 2.56	101
20	0	2.36 ± 1.94	84
	0.1	2.50 ± 2.39	90
40	0	2.19 ± 2.46	79
	0.1	2.08 ± 2.53	75

Data are means ± SD of 12–24 experiments.

and rat mesangial cells (27,28). As indicated in previous studies, the magnitude of the increase in sorbitol level and decrease in *myo*-inositol content in smooth muscle cells was related to the extracellular glucose concentrations. When smooth muscle cells were incubated with 30 mM glucose, sorbitol content increased rapidly, but *myo*-inositol levels decreased gradually. Morrison et al. (13,14) reported that elevated glucose concentrations caused an increase in sorbitol content and a reduction in oxygen diffusion in aortic intima-media preparations. Because the major element in aortic intima-media samples is intima smooth muscle, these results appear to reflect the properties of smooth muscle cells. Possibly, Morrison et al. (14) failed to detect a decrease in *myo*-inositol content because their incubation time was too short to permit detection of the slow decrease in *myo*-inositol content.

In most tissues, the intracellular *myo*-inositol concentration is many times that of the extracellular *myo*-inositol concentration (21,29). These high intracellular concentrations are maintained by the *myo*-inositol uptake mechanism and by the biosynthesis of glucose to *myo*-inositol. However, the activity of inositol-1-phosphate-syntase, the rate-limiting enzyme for *myo*-inositol synthesis, is very low; therefore, the effect of intracellular *myo*-inositol biosynthesis is likely to be less important in tissue that actively transports *myo*-inositol (30). Our study of *myo*-inositol uptake indicates that smooth muscle cells have at least two independent uptake mechanisms. One mechanism is directly dependent on extracellular *myo*-inositol concentrations, unsaturable uptake, and possibly simple

TABLE 5
Effects of 3-*O*-methyl-D-glucose and epalrestat on *myo*-inositol uptake

3-*O*-methyl-D-Glucose (mM)	Epalrestat (mM)	*myo*-Inositol uptake (pmol/1 × 10^4 cells)	Change from control (%)
0	0	2.78 ± 2.01	100
	0.1	2.72 ± 1.81	98
5	0	2.69 ± 1.83	96
	0.1	2.90 ± 2.53	104
20	0	2.71 ± 1.73	97
	0.1	2.62 ± 2.25	94
40	0	2.62 ± 2.32	94
	0.1	2.55 ± 2.04	92

Data are means ± SD of 12–24 experiments.

TABLE 6
Effects of 2-deoxy-D-glucose and epalrestat on *myo*-inositol uptake

2-deoxy-D-Glucose (mM)	Epalrestat (mM)	*myo*-Inositol uptake (pmol/1 × 10^4 cells)	Change from control (%)
0	0	2.78 ± 2.01	100
	0.1	2.72 ± 1.81	98
5	0	2.69 ± 2.01	97
	0.1	2.70 ± 1.90	97
20	0	2.41 ± 2.15	87
	0.1	2.44 ± 2.08	88
40	0	2.20 ± 2.42	79
	0.1	2.07 ± 2.53	74

Data are means ± SD of 12–24 experiments.

diffusion. The other mechanism is characterized by high-affinity, saturable uptake, ouabain sensitivity, and an energy-dependent transport mechanism related to Na^+/K^+-ATPase; active transport by carrier protein is probably also involved. At physiological *myo*-inositol concentrations (~30 μM) (31), *myo*-inositol uptake depends mainly on the active transport process (32). In our study, glucose-inhibited uptake related to active transport in a noncompetitive fashion, as demonstrated by Dixon plot analysis. In the presence of epalrestat, the failure of the Dixon plot to intersect for all values of the substrate provides evidence that intracellular sorbitol inhibits *myo*-inositol uptake. Substances with structures and molecular weights similar to glucose such as L-glucose, 3-*O*-methyl-D-glucose, 2-deoxy-D-glucose, sorbitol, and mannitol had no effect on *myo*-inositol uptake. Therefore, it is unlikely that glucose-related inhibition of *myo*-inositol is related simply to the structural similarity between glucose and *myo*-inositol or that extracellular mannitol and sorbitol failed to inhibit uptake simply because these compounds are not cyclic. Of the hexoses tested, only fructose reduced *myo*-inositol uptake, which is consistent with previous studies in other tissues (33,34), but it had only a slight effect. Thus, the depletion of *myo*-inositol content in our study appeared to be caused by the extracellular glucose-induced decrease in *myo*-inositol uptake, which maintains the intracellular *myo*-inositol level. Either epalrestat or statil reversed the depletion of *myo*-inositol induced by elevated glucose concentrations because these ARIs blocked the glucose-induced decrease in *myo*-inositol uptake. Previous studies have found that glucose reduced *myo*-inositol content and uptake in the rabbit peripheral nerve (5,32), mesangial cells (28), bovine endothelial cells (26,34), and retinal capillary pericytes (35). However, administration of an ARI reversed the decrease in *myo*-inositol uptake in the peripheral nerve (32), endothelial cells (26), and pericytes (35) but not in mesangial cells (28). Inhibition of *myo*-inositol uptake was competitive in peripheral nerve (34), mesangial cells (28), and endothelial cells (33) and noncompetitive in pericytes (35). Therefore, the type of inhibition may not be a useful measure of the effect of an ARI on *myo*-inositol uptake, although additional study is necessary to clarify this relationship. The effects of epalrestat on *myo*-inositol uptake in rabbit aortic smooth muscle cells exposed to glucose in this study were similar to the effects reported in bovine retinal capillary pericytes (35). In smooth muscle cells, glucose inhibited *myo*-inositol uptake noncompetitively, and the addition of ARIs resulted in recovery of the reduced *myo*-inositol content and reversal of the inhibition of *myo*-inositol uptake. Epalrestat alone and nonmetabolizable and partially metabolizable hexoses did not significantly inhibit *myo*-inositol uptake, suggesting that glucose inhibited *myo*-inositol uptake via the polyol pathway.

TABLE 7
Effects of substrates and drugs on *myo*-inositol uptake

Incubation conditions	*myo*-Inositol uptake pmol/1 × 10^4 cells	Change from control (%)
Control	2.78 ± 2.01	100
Insulin		
6 pM	2.80 ± 1.05	111
60 pM	2.74 ± 1.83	99
600 pM	2.68 ± 1.08	96
6000 pM	2.82 ± 1.40	101
Epinephrine		
1 μM	2.60 ± 1.25	94
10 μM	2.75 ± 1.27	99
Glucagon		
355 ng/L	2.92 ± 2.25	105
Fructose		
20 mM	2.02 ± 1.71	73
Sorbitol		
20 mM	2.42 ± 2.16	87
Mannitol		
20 mM	2.45 ± 1.32	88
Ouabain		
0.1 mM	1.73 ± 1.47*	62
1.0 mM	1.34 ± 1.42†	48
Cytochalasin B		
10 μM	2.03 ± 1.89	73

Data are means ± SD of 6–24 experiments.
*$P < 0.05$ vs. control.
†$P < 0.01$ vs. control.

Although epalrestat could not affect *myo*-inositol uptake in the absence of glucose or the presence of low glucose (5 mM) in our in vitro study (Table 2), Cohen et al. (36) reported that sorbinil, another ARI, increased Na^+/K^+-ATPase activity in isolated glomeruli of normal rat. Then, it is impossible to deny completely that epalrestat might directly stimulate Na^+/K^+-ATPase and improve its reduced activity. Additional study is required to clarify the mechanisms involved.

The increased sorbitol content, decreased *myo*-inositol level, and other metabolic abnormalities caused by exposure to high glucose may play an important role in the development of injury of smooth muscle cells, resulting in the characteristic atherosclerosis, e.g., Mönckeberg's calcific medial sclerosis in patients with diabetes. The use of an ARI may prevent these glucose-induced changes.

ACKNOWLEDGMENTS

We thank Prof. K. Kojima, Prof. S. Yoshida, and Dr. K. Koizumi of the Research Institute for Disease Mechanism and Control, Nagoya University School of Medicine, for reviewing the results and for preparing the place for

culture experiments of smooth muscle cells. The radioisotope study was conducted at the General Isotope Center, Tsuruma Branch, Nagoya University School of Medicine.

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